Biological Forum – An International Journal **14(2): 223-229(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Breeding of Parthenocarpic and Gynoecious Lines in Cucurbits and other Important Vegetable Crops

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(Received 21 January 2022, Accepted 30 March, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Parthenocarpic cultivar development is a viable breeding strategy. With a few backcrosses, the parthenocarpic characteristic can be passed from donor line to new types. The use of conventional breeding methods to develop parthenocarpic vegetables with desirable genes is highly effective. In addition, biotechnological tools can improve the efficiency and identification of parthenocarpic genes in crops, which can help with the development of parthenocarpic cultivars. In cucurbits, breeding for stable gynoecious lines is critical. Gynoecy is used to increase yield and reduce hybrid production cost. The major goal of the cucurbit breeding programme is to increase the number of female flowers while also stabilising gynoecious inbred parents. To generate gynoecious lines, isolate, self, and evaluate selfed progenies from original populations, and maintain these lines by using chemicals to induce staminate flowers for selfing. The use of gynoecious lines in hybrid development will increase output while lowering hybrid seed production cost. The use of parthenocarpy and gynoecism in crop production will not only increase the production but it will also add quality of the produce.

Keywords: Seedless fruit, Parthenocarpy, Gynoecious, Hormones, Sex expression.

INTRODUCTION

Parthenocarpy is the process of an ovary developing into a seedless fruit without pollination or fertilization. When a plant's fruits are completely empty of seeds, have a very small number of seeds, or contain aborted seeds, it is classified as parthenocarpic. Parthenocarpy could be used to produce a variety of vegetable crops during the winter months or, more broadly, to assure yield stability in the event of poor pollination conditions. A promising approach is to produce new cultivars with the ability to generate fruits without pollination or other artificial stimuli (Yoshioka *et al*., 2018).

Moreover, hybrid varieties of cucurbits are widely used in the production systems of many industrialised and developing countries. Cucurbit gynoecious lines are significant for hybrid seed production since the proportion of hybrid cultivars is constantly rising. Gynoecy is the condition where all flowering nodes produce only pistillate flowers. At present, several hybrid breeding programmes are focusing on stabilizing the gynoecious character and development of stable gynoecious lines (Chaudhary *et al*., 2001).

Parthenocarpy

Null (1902) coined the term parthenocarpy. The word parthenocarpy comprises of two words- Parthenos- Virgin and Carpy- fruit. The natural or artificial induction of fruit development without pollination and fertilization is known as parthenocarpy. An enhanced supply of phytohormones to the gynoecium from sources other than developing seeds has been observed to be adequate to trigger fruit formation in certain parthenocarpic plant varieties. This shows that parthenocarpic gene/s may predominantly alter hormone production, transport, and/or metabolism in the ovary, resulting in greater hormone levels capable of stimulating fruit formation without fertilization (Vivian-Smith *et al*., 2001).

Advantages of parthenocarpic vegetables

1. Due to adverse environmental condition, pollination is hampered, as result of which fruit production and productivity is affected, whereas, parthenocarpic vegetable does not need pollination and fertilization for fruit development.
2. **Novelty-** seedless tomato, parthenocarpic cucumber and seedless watermelon, as well as rise in vegetable shelf life for better preservation.
3. Improved vegetable fruit quality- absence of seeds in eggplant inhibits browning and pulp texture degradation (Maestrelli *et al*., 2003). Seeds can also produce chemicals that hasten the degradation of the fruit (watermelon and eggplant) (Dalal *et al*., 2006).
4. Seeds are undesirable for use in several vegetables due to their hard leathery texture, bitter flavour and

toxin content. As a result, substituting edible tissue for seeds is an appealing choice for consumers.

5. Seedless tomatoes have a better taste and have a higher TSS than seeded tomatoes, and parthenocarpic cucumbers produce early.

6. Providing seedless fruits and vegetables increased the profitability of processing industries.

7. Vertical fruit harvest- growing parthenocarpic cucumbers in greenhouses will result in increased profit due to continual fruit set on vine.

8. In parthenocarpic cucumbers, "crown set inhibition" has no impact, so fruit growth is continuous (Rao *et al*., 2018).

Parthenocarpic vegetables are classified into two types based on the nature of their origin: natural and artificial parthenocarpy.

Natural or genetic parthenocarpy

Obligatory- Genetic sterility has resulted in the development of seedless fruits. Coccinia and some cucumber genotypes naturally yield seedless fruits. E.g., ivy gourd.

Facultative- Due to poor pollination and fertilization conditions, seedless fruits are produced. In brinjal, natural parthenocarpy is only expressed when the weather is cold (7-10°C). This characteristic is facultative since it is only expressed in cold temperatures; once pollination temperature is favourable, normal fruit and seed set occurs. E.g., tomato, brinjal

Genetics of parthenocarpy

The mode of inheritance for parthenocarpic fruit has been observed in nature and in several species, and it ranges from a single gene to several quantitative trait loci either dominant or recessive. E.g., tomato, capsicum, muskmelon (single recessive gene), eggplant (single dominant gene), cucumber (incomplete dominant gene Pc) and summer squash (single gene with incomplete dominance) (Rao *et al*., 2018).

Artificial parthenocarpy

Plant hormones are applied externally, irradiation pollen is used, or biotechnological techniques are used to generate artificial parthenocarpy (Tiwari *et al*., 2012). Exogenous application of irradiation pollen, natural or synthetic auxins and gibberellins during ovary development increases IAA contents, resulting in fruit growth without fertilization.

Methods to induce parthenocarpy

1. Environmental factors: High or low temperature, humidity, low light intensity, heavy rain and strong wind are all factors, which adversely affect the several steps of the reproductive process. The most prominent of these is temperature stress, which causes parthenocarpic fruit development in several vegetable crops. Parthenocarpy is induced by both high and low temperatures. Low temperature (Freezing temperature < 5°C) promotes parthenocarpic fruit in tomato, summer squash, bell pepper, and eggplant (7 to 10°C) (Fuzhong *et al*., 2005). In tomatoes, a high temperature (32/26°C) causes the formation of parthenocarpic fruit and an underdeveloped bloom. Under high temperatures, there was also a slow transition of undeveloped flowers to parthenocarpic fruit (Sato *et al*., 2001). Other environmental factors like low irradiance, severe water stress, and high irradiance have also been reported to induce parthenocarpy in vegetables.

2. Plant growth regulators: Exogenous application of plant growth hormones such as auxins, cytokinins, and gibberellins in vegetable crops may result in the formation of parthenocarpic fruits (Dhatt and Kaur, 2016). Gustafson (1936) was the first to disclose the application of exogenous auxin to a flower for the induction of parthenocarpy. Exogenous auxin induces parthenocarpic fruit by activating auxin-biosynthetic genes in the ovaries and ovules. As DELLA silencing also produces facultative parthenocarpy in tomato fruits, auxin upregulates gibberellin production and increases GA-dependent degradation of DELLA proteins (Marti *et al*., 2007). The apical stalk serves as a source of inhibitors, inhibiting fruit growth even in the absence of a stimulus such as pollination. Plant decapitation redirects GAs from mature leaves to the unpollinated ovary, causing the parthenocarpic cycle to be stimulated.

Table 1: Plant growth regulators for parthenocarpic fruit development.

Crop	Growth regulator	Stage of treatment	Type of parthenocarpy	References
Tomato	CPPU @ 100mg/l, GA3 @200mg/l	At anthesis	100% seedless fruit set	Jiagang *et al.*, (2013)
Brinjal	GA3 @ 2700ppm 2, 4-D @2.5ppm (foliar spray)	At anthesis	GA3 induced completely seedless fruits; The degeneration of seeds was triggered by2, 4-D.	Rao *et al*., (2018)
Cucumber	GA3 @ 100mg/l	Pre-anthesis	Parthenocarpy	Rao *et al*., (2018)
Watermelon	CPPU @ 100-200 ppm	At anthesis	Complete parthenocarpy	Kawamura *et al*., (2018)
Pumpkin	GA3 @ 150ppm	Prior to anthesis	96.9% seedless fruit	Hossain, (2015)
Teasle gourd	2,4-D@ 50-100ppm, GA3@ 100ppm	At anthesis	Complete parthenocarpy	Rasul *et al*., (2008)

3. Mutation: Natural mutations occur in nature and are exploited in traditional breeding programmes. The parthenocarpic sha-pat mutants in the tomato line 'Montfavet 191' are a good illustration of this. Radiation treatments such as helium accelerated ions in tomato, soft-X-ray in watermelon (Kawamura *et al*., 2018) and gamma irradiation in *citrullus lanatus* (Sugiyamma and Morishita, 2002) have all been successful in generating parthenocarpic mutants.

4. Polyploidy: To produce parthenocarpic fruit, scientists used an unbalanced embryo and endosperm development on a triploid background. F1 hybrid plants generated from a cross between tetraploid and diploid parents produce seedless watermelon fruits with just residual integuments (Kihara, 1951). Similary, Habashy *et al*., (2004) reported triploidy in tomato and cucumber by crossing diploid with tetraploid as a result of increased dry matter, TSS in tomato and Long fruiting period (2-3 months), and the ability to tolerate high temperatures (45°c) in cucumber. In wide crossings, chromosome removal may result in the development of haploids, which are of great interest to breeders.

5. Conventional breeding:

Two fundamental steps in conventional breeding:

— Creating a breeding population that segregates for the parthenocarpic trait of one parental genotype

— Selecting individuals progeny from a segregated population with parthenocarpy and favourable non-parthenocarpic parent characteristics

Intraspecific hybridization has been used to create a variety of parthenocarpic lines, such as tomatoes: Oregon T5-4, Oregon cherry, Oregon 11, Line 75/79, and IVT-line 2; eggplant: AE-P01, Talina2/1. The parthenocarpic eggplant cultivar "anominori" was developed as an F1 hybrid between two inbred lines, AE-P08 and AE-P01. AE-P08 was selected from the cross between F3 plant derived from Nakate Shinkuro × Talina and F4 plant derived from Talina × Nasu Chuukanbohon Nou 1. AE-P01 was selected from the cross between Talina and Nasu Chuukanbohon Nou 1 (Saito *et al*., 2009). Interspecific hybridization is the most popular method for obtaining parthenocarpic fruits in a variety of crops, such as watermelon.

Solanum esculentum × *Solanum peruvianum* (Lesley and Lesley, 1941)

L. esculentum × *L. hirsutum* -Severianin (Philouze and Maisonneuve, 1978)

Solanum habrochaites x *Solanum lycopersicum*- IVT-line 1 (Zijlstra, 1985)

6. Biotechnological approaches: Pollination for fruit development is replaced by increased quantities of hormones in the ovary or ovules, which has been exploited utilising biotechnological methods. The various approaches to increasing auxin levels in the ovary yielded good results in parthenocarpy induction (Gorguet *et al*., 2005). Seedless fruits can be created by expressing the auxin synthesising gene iaaM from *Pseudomonas syringae* pv. *savastanoi* under the control of the ovule/placenta specific promoter from the DefH9 gene of *Antirrhinum majus*. Transgenic approaches and gene silencing are effective techniques for interfering with the expression of phytohormone-synthetizing genes involved in parthenocarpic fruit development. Transfer of the rolB gene in tomato regulates auxin synthesis, resulting in seedless fruit formation (Nancy, 2015). Downregulating Chalcone synthase (Chs), the first gene in the flavonoid biosynthesis pathway, has also been used to produce seedless fruits (Schijlen *et al*., 2007). Gene AUCSIA action silenced by using gene silencing techniques as result auxin response increase in tomato and developed parthenocarpic fruit (Molesini *et al*., 2009). Biotechnology provides a wider range of options and makes obtaining parthenocarpic cultivars easier than traditional breeding.

Members of the Cucurbitaceae family have staminate, pistillate, and hermaphrodite flowers, resulting in the emergence of monoecious, gynoecious, trimonoecious, andromonoecious, gynomonoeciuos, gynodioecious, androecious and dioecious sex forms. Sex manipulation is the technique of changing the male-to-female flower ratio within individuals of a species, which leads to changes in the flowering phenology sequence in order to increase economic yield. In cucurbit breeding, sex inheritance is crucial, and sex expression is regulated mostly by genetic and environmental variables (Megharaj *et al*., 2017).

Sex Manipulation

Controlling non-genetic factors such as environmental influences, cultural traditions and plant growth regulators can easily modify sex expression in cucurbits.

Role of environmental effects on sex expression

Low temperature, short photoperiod, and high moisture availability favour female sex expression. Male flowers are produced due to the high temperature and long photoperiod. Due to endogenous ethylene synthesis under short-day conditions (8 h photoperiod), female flower producing nodes increased threefold in andromonoecious cucumbers and sevenfold in monoecious cucumbers (Yamasaki *et al*., 2003).

Role of cultural practice on sex expression

Irrigation, fertilizer application, and planting season all have a modest influence on sex manipulation. For example, increased nitrogen application results in more vegetative growth, which reduces the reproductive stage and causes flowering to be postponed. Mineral nutrients may influence plant sex expression via hormonal balance. Many cucurbits have sex expression influenced by mineral elements like boron. Application of boron at 4 ppm reduces number of days to first male and female flower production in cucumber and watermelon (Singh and Choudhary, 1988). Boron at 4ppm reduces the number of days it takes for the first male bloom to appear in bittergourds, as well as the male: female flower ratio (Gedam *et al*., 1998).

Role of plant growth regulators on sex expression

Growth regulators applied during the two-or four-leaf stage, which is the key stage at which either sex can be suppressed or promoted, it can change the sex ratio and sequence. Plant hormones also influence the male-to-female flower ratio in cucumber plants; ethylene and auxin encourage female flower formation, whereas gibberellins stimulate male flower formation. Ethylene suppresses gibberellins, a hormone that causes male flower formation and hence helps induce female flowers. GA_3 as an ethylene biosynthesis blocker and $AgNO_3$ as an ethylene action blocker prevent ethylene action and boost male flower production.

Development and Maintenance of gynoecious lines

The desire to stabilise the gynoecious character and generate stable gynoecious inbred parents has grown in recent years, and it has been a frequent goal of many hybrid breeding initiatives. The development of gynoecious lines is mainly due to the involvement of spontaneous or chance segregation of gynomonoecious lines, which lead to the isolation of gynoecious lines in the segregating population. Repeated backcrossing improves gynoecious lines and can be maintained via selfing. It is possible by utilising growth regulators to induce staminate flowers for selfing as a pollen source (Chaudhary *et al*., 2001). Bommesh *et al*., 2020 develop and maintain gynoecious inbred lines from the gynoecious parthenocarpic cucumber hybrids. To develop segregating populations, four slicing cucumber lines were raised. Individual plant selection was made in the F2 population. Based on the gynoecy sex expression, these lines were forwarded to the F4 generation, and the average performance of all four gynoecious lines was recorded.

Gynoecious lines are developed through inbreeding and plant-to-row selection; complete gynoecious development take 3-4 consecutive selfing generations, and silver nitrate ($AgNo_3$) was found to be statistically significantly superior for effective staminate flower induction for the maintenance of gynoecious lines among the chemicals used. In bittergourd, four gynoecious lines (Gy-1, Gy-14, Gy-15, and Gy-34) were isolated as 100% gynoecious in nature (Jadhav *et al*., 2018).

Maintenance of gynoecious lines

The following are common guidelines for inducing male flowers in gynoecious line:

• At the beginning when the plants have two true leaves, three applications of GA3 at 1000 ppm are sprayed at 15-day intervals.

• From the second leaf stage, three applications of GA4/7 at 50 ppm at fortnightly intervals.

• Before the first blossom opens, spray with a 600 ppm silver nitrate solution.

• At the two and four true leaf stages, use $AgNO_3$ at 250-300 ppm or silver thiosulphate at 400 ppm to encourage male flowering.

Timeline of gynoecism

• The first gynoecious line (M.SU.713-5) in the Korean cucumber cultivar 'Shogoin' (PI220860) was reported by Peterson and Anhder (1960).

• In cucumber, More and Seshadri (1988) identified four tropical gynoecious lines: 87-304-6, 87-316, 87-319-12 and 87-338-15.

• In muskmelon, a stable gynoecious line (WI998) was produced from monoecious x hermaphrodite crosses (Peterson *et al*., 1983).

• Three gynoecious lines, 86-104, 86-105, and 86-118, have been produced for true breeding gynoecious sex in muskmelon, according to (More *et al*., 1987).

• A gynoecious line known as Gy263B has been discovered in bitter gourd (Ram *et al*., 2006).

• IARI developed two gynoecious lines (DBGy-201 and DBGy-202) from a wild population (Behera *et al*., 2006).

• In bitter gourd, two gynoecious lines (IIHRBTGy-491 and IIHRBTGy-492) have been discovered (Varalakshmi *et al*., 2014).

• Watermelon with a gynoecious line has been discovered (Jiang and Lin, 2007).

Development of different gynoecious lines in cucurbits by IARI

Development of tropical gynoecious lines in cucumber

In temperate locations, Munger (1979) found that the gynoecious lines Gy14, SR551F, Gy3, Gy57, and Table Green 68 are best for producing F1 hybrids in slicing and pickled cucumbers. Unfortunately, with the high temperatures and long photoperiodic conditions encountered in tropical producing zones, those lines have been proven to be unstable for gynoecy. As a result, there is a need to develop gynoecious lines, which are suitable for tropical production. Temperate gynoecious lines (Gy14, SR551F, Gy3, Table green 68x Gy 3 F2, Wisconsin2757) were crossed with tropical monoecious lines (Gy14, SR551F, Gy3, Table green 68x Gy3F2, Wisconsin 2757) (Poona Khira, RKS296, RKS300). Silver-nitrate was used to maintain gynoecious aggregates (250 ppm, twice). As a result, several tropical gynoecious lines have been isolated, and they are now in the F4 or F5 generation.

During the summer and rainy seasons of 1987, four lines were found to be true-breeding gynoecious lines: 87-304-6, 87-316, 87-319-12, and 87-338-15 (More and Seshadari, 1988).

Development of gynoecious lines in muskmelon (Peterson *et al*., 1983) developed Wisconsin998 (WI 998), a stable gynoecious line. Three gynoecious lines namely 86-104, 86-105, and 86-118, have been produced for true breeding gynoecious sex at the Indian Agricultural Research Institute in New Delhi. The 86-104 and 86-105 lines were developed by crossing a gynomonoecious line with hermaphrodite-2, whereas the 86-118 lines were developed by crossing Monoecious-1 with hermaphrodite-1 (EC 70674).

This report's gynoeocious lines are all F6 generation. As a result, the parents were kept in a purely gynoecious state. The gynoecious stability and maintenance scores were reached in the same growth season this way. Later generations sprayed selected lines with 400 ppm STS at two true leaf stages, resulting in beautiful flowers that were used to perpetuate gynoecious stock (More *et al*., 1987).

Inheritance of gynoecism in cucurbits

The sex expression in the progeny seeds of female homosexual crosses produced 100% female plants in F1, indicating a female:male sex ratio of 1:0, whereas 1:1 segregation of male and female plants in heterosexual crosses suggested that xx/xY chromosomes control sex expression. (Jiang and Lin, 2007) found the gynoecious gene (gy) in watermelon and produced a normal monoecious F1 hybrid by crossing a gynoecious line (Gynoecious 1) with a monoecious line (A123). In F2, the ratio of monoecious to gynoecious sex forms was 86:18, and progeny backcrosses led to segregation, with a ratio of 59:43, indicating that gynoecism in watermelon is controlled by a single recessive gene. The inheritance of gynoecism in bitter gourd was studied using the GY263B gynoecious line and the Pusa-Do-Mousami monoecious line, with the phenotype of F1 revealed to be monoecious, and the gynoecious in bitter gourd is controlled by a single recessive gene, according to the F2 population and testcross (Ram *et al*., 2006). Inheritance of gynoecism in cucumber is governed by a single dominant gene (Acr/acr) (Pati *et al*., 2015) and inheritance of gynoecism in ridge gourd is by a major recessive gene (Singh *et al*., 2012).

Use of gynoecious lines for hybrid seed production

Cucumber

Without the need for male flower emasculation, hybrid seeds can be produced by using gynoecious maternal lines. There are different systems that have been proposed to produce hybrid cucumber seeds by using gynoecious lines: Gynoecious × Monoecious, Gynoecious × Gynoecious, Gynoecious × Hermaphrodite and Gynoecious × Andromonoecious hybrids.

From these systems, **Gynoecious × Monoecious** and **Gynoecious × Gynoecious hybrids** are most commonly used**.**

Gynoecious × Monoecious

For the production of stable inbred lines, this approach has become popular. The cross of gynoecious and monoecious lines produced a hybrid with high female sex expression, uniform and concentrated fruit production, and hybrid vigour.

1. Planted female and male parents (Gynoecious and Monoecious line) in ratio of 1:3 and maintained isolation distance.
2. Pollination is performed by bees.
3. Seeds are collected only from gynoecious plants that are hybrid seeds and also fruits of monoecious plant harvested which kept for maintenance.

Gynoecious × Gynoecious

After one parent has been treated with a growth regulator to encourage male flower production, two gynoecious lines are crossed. When compared to hybrids created by combining gynoecious and monoecious lines, these hybrids are more stable for gynoecious sex expression.

Although hybrid seed produced in this manner can be costly, the expense may be justified for greenhouse cucumbers due to their high value and low seed requirements.

Muskmelon

Use of gynoecious line (WI 998) for hybrid production and maintained by inducing male flowers using Silver nitrate/ Silver thiosulphate sprays @ 300-500ppm at 2-4 true leaf stage.

Bitter gourd

Two gynoecious lines **(DBGy-201 and DBGy-202)** have been developed from natural populations at IARI (Behera *et al*., 2006). DBGY-201 × Pusa Vishesh showed the highest heterosis for earliness, and hybrid DBGY-202 × Priya was reported to have the maximum heterosis for fruit length, weight, and yield (Dey *et al*., 2009).

Achievements

Sr. No.	Crop	Hybrid	Remark
1.	Cucumber	Pusa Sanyog	(Japanese gynoecious line × Green Long Naples)
		DCH-1 and DCH-2	Tropical gynoecious lines developed at IARI, New Delhi
		KTCH-11	prolific-bearing hybrid, developed at IARI Regional Station, Katrain
		PH-9	GYC-2 × Phule Shubhangi, (MPKV, Rahuri)
		PH-18	GYC-4 × Poona Khira, (MPKV, Rahuri)
		KH-1 and KH-2	Developed at YSP University of Horticulture and Forestry, Solan
2.	Muskmelon	MH-10	(WI 998 x Punjab Sunehri), developed at PAU, Ludhiana.
3.	Bittergourd	Pusa Hybrid-4	First gynoecious based hybrid, (IARI New Delhi)

CONCLUSION

Parthenocarpy is a significant characteristic for enhancing vegetable crop yield, quality and processing. Parthenocarpic vegetables can be created naturally or by using several approaches such as plant growth regulators, conventional breeding, mutation, irradiated pollen, chromosomal number alternation and biotechnological tools. Biotechnological tools can be used to improve the efficiency and identification of parthenocarpic genes across crops, which will benefit humanity. In cucurbitaceous vegetables, gynoecious lines behave as male sterile lines. The production and maintenance of stable gynoecious inbreds in cucurbits will make the hybrid development cost effective.

FUTURE SCOPE

Parthenocarpy is a highly profitable trait in a variety of horticultural crops. Parthenocarpic plants can set and develop fruits under unfavourable environmental conditions. The parthenocarpic can be very well exploited under protected conditions. Cost of hybrid seed production especially in cucurbits can be made cost effective with the use of gynoecious lines. Moreover, gynoecism will impart more number of female flowers which in term will increase the female to male flower ratio. So production can be increased to a greater extend with the use of gynoecious lines.

Acknowledgment. I wish to express my sincere gratitude to Dr. B.S. Dogra, Department of Vegetable Science, for providing me an opportunity to write this review article. I sincerely thank Dr. Shiv Pratap Singh for his encouragement in carrying out this review article. I also wish to express my gratitude to the professors and other staff members of the College of Horticulture and Forestry Neri, Hamirpur (H.P.) who rendered their help during the period of my work.

Conflict of Interest None.

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How to cite this article: Riya Rani, B.S. Dogra and Shiv Pratap Singh (2022). Breeding of Parthenocarpic and Gynoecious Lines in Cucurbits and other Important Vegetable Crops. *Biological Forum – An International Journal, 14*(2): 223-229.